Phytoplankton biomass and size structure in Xiangshan Bay, China: Current state and historical comparison under accelerated eutrophication and warming

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\textbf{ABSTRACT}

To explore the effects of coastal eutrophication and warming on phytoplankton biomass and cell size, we analyzed current and historical data for size-fractionated chlorophyll \textit{a} (chla) in Xiangshan Bay, China. Results showed that micro- and nanophytoplankton overwhelmingly dominated (> 84%) in all seasons. The contribution of micro-chla was significantly lower in warm than in cold seasons, whereas contribution of pico-chla showed the opposite result. Overall, the micro-chla contribution increased with decreasing pico-chla contribution from the stable, clear, eutrophic upper bay to the turbulent, turbid lower bay, indicating that phytoplankton size structure on a spatial scale was largely shaped by water column stability and light rather than by nutrients. Since the 1980s, phytoplankton biomass, primary productivity, and micro-chla contribution in Xiangshan Bay have increased sharply with increasing nutrient amounts and temperature. Additionally, it seems that algal bloom seasonality has shifted forward from spring to winter since the power plant operations in 2006.

\section{1. Introduction}

Cell size is a crucial ecological trait of phytoplankton. Phytoplankton cell size influences metabolic activity, nutrient uptake and requirements, sinking, diversity, and grazing, all of which contribute considerably to local primary production and biogeochemical processes (Stolte and Riegman, 1995; Litchman et al., 2007; Finkel et al., 2010; Marañón, 2015). Moreover, cell size is directly related to dynamics of food webs and trophic structure: shorter, herbivore food webs prevail in systems dominated by large-celled phytoplankton, whereas longer, microbial food webs are based on smaller cells (Legendre and Rassoulzadegan, 1996; Mousseau et al., 1996; Guenther et al., 2008). Therefore, understanding the current state and long-term variation of phytoplankton size structure is critical in biological oceanography (Marañón, 2015).

Warming and eutrophication have emerged as the most severe environmental problems in coastal ecosystems worldwide, with variation in phytoplankton size scaling usually related to temperature and nutrients. Numerous mesocosm experiments have shown that warming reduces phytoplankton cell size directly by increasing metabolic rate or indirectly by size-selective grazing and enhanced nutrient limitation (Somer et al., 2012; Winder and Sommcr, 2012; Peter and Sommer, 2013, 2015; Yvon-Durocher et al., 2015). Long-term field observation in southern Caribbean Sea revealed that increased temperatures favored small-sized phytoplankton through reduced upwelled nutrients (Taylor et al., 2012). However, warming in high-latitude seas associated with higher light utilization and productivity favored large cells (Marañón, 2015). Large-celled phytoplankton has a competitive advantage over small cells in coastal eutrophic waters (Agawin et al., 2000; Irwin et al., 2006; González-García et al., 2018), being able to sustain higher specific nutrient uptake rates (Irwin et al., 2006), intracellular nutrient pools (Stolte and Riegman, 1995), and photosynthetic efficiency (Cermeño et al., 2005; Marañón et al., 2007). Although warming and eutrophication drastically affect phytoplankton community and harmful algal blooms (HABs; Hallegraeff, 2010; Anderson et al., 2012; Jiang et al., 2014; Xie et al., 2015; Xiao et al.,...
2018), little is known about their complex interactions with phytoplankton size structure in coastal waters (Agawin et al., 2000).

Xiangshan Bay (XSB) is an eutrophic, semi-enclosed subtropical bay located in Ningbo, China. It is a long (~60 km), narrow (~3–8 km) bay associated with the East China Sea. The water-residence times in the upper, middle, and lower sections are approximately 80, 60, and 15 days, respectively, for 90% water exchanges (Ning and Hu, 2002). Previous studies on XSB have shown apparently seasonal and spatial changes in size-fractionated (> 20 and < 20 μm) chlorophyll a (chl; Liu et al., 1997, 1998; Ning and Hu, 2002). However, the distribution patterns and controlling factors of phytoplankton size structure (> 20, 2–20, and < 2 μm) remain poorly documented. Physicochemical processes in XSB, including nutrient pulse, sediment resuspension, and thermal discharge from power plants, exhibit significant spatial and seasonal changes (Ning and Hu, 2002; Yang et al., 2018; Jiang et al., 2019). On the basis of previous studies on cell-size tradeoffs (Irwin et al., 2006; Key et al., 2010; Cloern, 2018), we hypothesized that seasonal and spatial changes in phytoplankton biomass and size structure in XSB are largely regulated by nutrients, temperature, and light.

Since the 1980s, XSB has been subjected to extensive anthropogenic activities, including severe eutrophication driven by industrial and agricultural sewage discharge and excessive mariculture (Ning and Hu, 2002; Jiang et al., 2012, 2013a; Yang et al., 2018). Additionally, the Ninghai and Wushashan Power Plants located in the inner bay (Fig. 1) began operating in December 2005 and March 2006, respectively, with large volume of thermal discharge (Jiang et al., 2013b), potentially exacerbating surface temperature increase in XSB. However, how phytoplankton biomass and size structure respond to accelerated eutrophication and warming remains unclear. Chen et al. (2012) found that warming may enhance phytoplankton loss to microzooplankton herbivory in eutrophic waters, indicating an increased dominance of microphytoplankton under predator–prey interactions. Long-term observations in Daya Bay, South China Sea, where a nuclear power station is situated, suggested that dominant species shifted from diatoms to dinoflagellates (e.g., Scrippsiella trochoidea and Ceratium spp.) in warmer waters caused by thermal discharge (Li et al., 2011; Wu et al., 2017). Additionally, dominance and HAB occurrence of large-celled dinoflagellates increased sharply with increasing temperature and nutrients after a nuclear power station commenced operations (Yu et al., 2007; Wu et al., 2017). Marañón (2015) suggested that increased nutrient input in coastal regions promotes the occurrence of large-sized phytoplankton blooms and thus a shift toward larger mean cell size, even in warming waters. In this context, we hypothesized increased microphytoplankton contribution in XSB under exacerbated eutrophication, increased large-celled dinoflagellates, and higher temperatures, in conjunction with enhanced microzooplankton grazing.

To test our hypotheses, we conducted four cruises in XSB in different seasons during 2015 and subsequently compared with historical data that we obtained since the 1980s. Using a size-fractionated chl-a method, we examined how changes in environmental variables influence phytoplankton biomass and size structure. Our objectives were 1) to reveal the spatial and seasonal distribution and controlling factors of size-fractionated phytoplankton and 2) to explore long-term changes in phytoplankton biomass and size structure under increased eutrophication and warming.

2. Materials and methods

2.1. Study area and sampling stations

XSB is adjacent to the Changjiang Estuary and Hangzhou Bay (Fig. 1) and has three branched bays, including Tie, Huangdun, and Xihu Harbors. Under the East Asian monsoon, XSB is subjected to the southward flowing, turbid, inshore Zhemin Coastal Current in cold/dry seasons and the northward flowing, clear, offshore Taiwan Warm Current in warm/wet seasons. Winter and spring were defined as cold and summer and autumn as warm seasons according to SST variation. Tides in XSB are semi-diurnal with an average tidal range of 3.3 m (Editorial Committee of the Bay Chorography in China (ECBCC), 1992). The tidal range increases with decreasing tidal current velocity from the lower bay with fine sediment to the upper bay with coarse sediment. Therefore, the lower bay shows strong wind- and tide-induced vertical mixing and sediment resuspension resulting in high turbidity, whereas the upper bay has high transparency and water column stability (ECBCC, 1992; Jiang et al., 2013b). The precipitation decreased from the upper to the lower bay and the annual precipitation in Ningbo was 1989 mm during 2015, considerably higher (37%) than the multi-year average (Ningbo Meterological Bureau (NMB), 2016). Moreover, the precipitation was significantly higher in warm than in cold seasons, resulting in lower salinity in warm than in cold seasons. Freshwater inputs from a total of 95 rivers around XSB, particularly in the upper and middle sections (ECBCC, 1992), resulting in low salinity and high nutrients in the inner bay under low water exchange (Jiang et al., 2013b). Additionally, nutrients originated from extensive mariculture, including fish cages and shrimp ponds, exacerbate eutrophication in the inner bay (Ning and Hu, 2002; Yang et al., 2018).

Fig. 1. Sampling stations and sectional divisions (upper, middle, and lower) in Xiangshan Bay (XSB). TH: Tie Harbor; HDH: Huangdun Harbor; XHH: Xihu Harbor; NPP: Ninghai Power Plant; WPP: Wushashan Power Plant.
A total of 25 stations were established for testing in each season (Fig. 1), with XSB divided into upper (stations 1–6, 19, and 20), middle (stations 7–13, 21, and 25), and lower (stations 14–24) sections. Four cruises were conducted in January (winter), April (spring), July (summer), and October (autumn) during 2015. In this subtropical bay, December–February, March–May, June–August, and September–November are usually defined as winter, spring, summer, and autumn, respectively (ECBCC, 1992; Ning and Hu, 2002). Therefore, the present investigation month is typical for each season.

### 2.2. Sample collection

Surface (0.5 m depth) and bottom (0.5 m above seabed) water samples from each station were collected in 5-L Niskin bottles. Temperature and salinity were measured using a YSI model 30 salinity meter, and transparency was determined using a Secchi disc. Water samples for dissolved inorganic nitrogen (DIN), phosphorus (DIP), and silica (DSi) analysis were filtered through a 0.45-μm cellulose acetate filter, and saturated HgCl2 solution was subsequently added for further analysis. Nutrient concentrations were determined using colorimetric methods according to the GB 17378.4-2007. For suspended solids analysis, after samples were filtered through preweighed GF/C filters (Whatman), filters were dried at 105 °C to a constant mass and weighed. Dry mass of particles retained on each filter was calculated by subtracting the filter mass from dried mass. The data on transparency, suspended solids, temperature, salinity, and nutrients were already reported (Jiang et al., 2019). Mesozooplankton (>160 μm) abundance data were derived from Du (2018).

Water samples (100 mL) for chla were size-fractionated into micro- (>20 μm), nano- (2–20 μm), and picophytoplankton (<2.0 μm) by using three types of filters. First, water was filtered using a 0.2 μm nylon filter (Millipore), followed immediately by 2-μm polycarbonate (Millipore) and 0.7-μm GF/F filters (Whatman). The size-fractionated chla retained by the filters was analyzed using a Turner Design fluorometer after extraction in 90% acetone for 24 h at −20 °C. Pheophytin (pheo) was measured after addition of two drops of 5% HCl. Pheopigments are chlorophyll degradation products that are diagnostic of phytoplankton mortality through herbivory, autolysis, decomposition, viral lysis, and sediment resuspension (Lorenzen, 1967); pheo/chla thus indicates phytoplankton succumbing to predation, senescence, and decomposition (Taylor et al., 2012; Chen et al., 2015).

### 2.3. Historical data collection

To ascertain the warming trend in XSB, the monthly mean SST during 2003–2017 was derived from MODIS-Aqua Level 3 Product-Monthly SST4 (4 μ nighttime with 4 km resolution; https://oceancolor.gsfc.nasa.gov/l3). Data of chla, primary productivity, size structure, and nutrients in XSB were derived from the literatures (ECBCC, 1992; Gu and Xu, 1993; Liu et al., 1997, 1998; Ning and Hu, 2002; Huang et al., 2008; You and Jiao, 2011; Jiang et al., 2013b; Wang et al., 2013; Shou and Zeng, 2015). These historical data were not all from the same stations, but covered the entire XSB from the upper to the lower bay. Therefore, the error between previous and present data collected from different stations could be reduced to a minimum. Additional measurements of size-fractionated chla in XSB were conducted in four seasons during 2017 because of the limited data of size structure change from 1992 to 2015. The sample collection and determination methods of chla were consistent with those in 2015.

### 2.4. Data analysis

All statistical tests were performed using open source software R v.3.5.1 (R Core Team, 2018; https://www.R-project.org). A Kruskal–Wallis test showed no consistent difference in size-fractionated chla concentrations between surface and bottom layers in all seasons, which indicated an even vertical distribution of phytoplankton biomass in the XSB. For ease of comparison and analysis, we defined the mean value of surface and bottom chla concentrations as the depth-averaged chla concentration at a certain station in this study according to Jiang et al. (2019). The same method was also applied for environmental variables excluding transparency and suspended solids. A Kruskal–Wallis test was performed to reveal significant differences in environmental parameters and size-fractionated chla in different sections (upper, middle, and lower), seasons, and years. Spearman’s rank correlation was used to determine the relationship between environmental variables and size-fractionated chla concentration and contribution. The SST data were analyzed for the presence of monotonic increasing trends by the Mann-Kendall test using the package “Kendall” in software R v.3.5.1.

### 3. Results

#### 3.1. Current environmental parameters

All environmental parameters in XSB showed significant (p < 0.01) seasonal differences (Table 1). Transparency was significantly (p < 0.05) lower in spring than in other seasons, whereas suspended solids showed the opposite result. Temperature, DIP, DSi, and Si/N were significantly (p < 0.01) lower in cold than in warm seasons, whereas salinity and N/P showed the opposite results. DIN varied significantly (p < 0.01) among seasons, showing summer > winter and spring > autumn.

The average water depths in the upper, middle, and lower bay were

### Table 1

Comparison of environmental variables, size-fractionated chlorophyll a (chla; μgL−1), and pheophytin/chla (pheo/chla) among seasons in XSB by using the Kruskal–Wallis test. Superscripted lower-case letters within the same row indicate significant difference (p < 0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparency (m)</td>
<td>0.8 ± 0.40b</td>
<td>0.6 ± 0.40a</td>
<td>1.1 ± 0.30b</td>
<td>1.0 ± 0.50a</td>
</tr>
<tr>
<td>Suspended solids (mg/L)</td>
<td>20.4 ± 29.3</td>
<td>69.7 ± 55.2</td>
<td>45.9 ± 74.2a</td>
<td>57.1 ± 101.0a</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10.3 ± 6.6</td>
<td>15.7 ± 12.6</td>
<td>27.1 ± 14.4</td>
<td>24.0 ± 5.0</td>
</tr>
<tr>
<td>Salinity</td>
<td>24.7 ± 1.5</td>
<td>26.1 ± 0.65</td>
<td>20.2 ± 0.96</td>
<td>21.2 ± 1.64</td>
</tr>
<tr>
<td>DIN (μmol L−1)</td>
<td>59.01 ± 11.85a</td>
<td>55.60 ± 2.21a</td>
<td>67.93 ± 5.11</td>
<td>53.10 ± 10.43</td>
</tr>
<tr>
<td>DIP (μmol L−1)</td>
<td>1.47 ± 0.39a</td>
<td>1.29 ± 0.23a</td>
<td>1.86 ± 0.41b</td>
<td>1.90 ± 0.54</td>
</tr>
<tr>
<td>DSi (μmol L−1)</td>
<td>42.66 ± 4.03a</td>
<td>41.75 ± 4.12a</td>
<td>69.90 ± 8.43b</td>
<td>59.54 ± 10.34</td>
</tr>
<tr>
<td>N/P</td>
<td>40.8 ± 3.11</td>
<td>43.9 ± 5.4</td>
<td>37.6 ± 5.2</td>
<td>28.6 ± 2.4</td>
</tr>
<tr>
<td>Si/N</td>
<td>0.74 ± 0.10a</td>
<td>0.75 ± 0.06</td>
<td>1.03 ± 0.09b</td>
<td>1.13 ± 0.06</td>
</tr>
<tr>
<td>&gt;20 μm chla (μgL−1)</td>
<td>0.60 ± 0.37</td>
<td>0.44 ± 0.12</td>
<td>0.27 ± 0.21b</td>
<td>0.67 ± 0.61a</td>
</tr>
<tr>
<td>2–20 μm chla (μgL−1)</td>
<td>1.04 ± 0.81a</td>
<td>0.87 ± 0.34</td>
<td>1.85 ± 1.32b</td>
<td>1.31 ± 0.84</td>
</tr>
<tr>
<td>&lt;2 μm chla (μgL−1)</td>
<td>0.18 ± 0.25</td>
<td>0.10 ± 0.07</td>
<td>0.38 ± 0.29a</td>
<td>0.37 ± 0.34</td>
</tr>
<tr>
<td>Total chla (μgL−1)</td>
<td>1.83 ± 2.33</td>
<td>1.41 ± 0.44</td>
<td>2.50 ± 1.64b</td>
<td>2.35 ± 1.49</td>
</tr>
<tr>
<td>Pheo/chla</td>
<td>0.67 ± 0.75</td>
<td>1.15 ± 0.91a</td>
<td>0.53 ± 0.35</td>
<td>0.56 ± 0.47</td>
</tr>
</tbody>
</table>
12.5, 15.0, and 13.0 m, respectively. Environmental parameters differed significantly \((p < 0.01)\) among sections in most cases (Fig. 2). Overall, transparency decreased significantly with increasing suspended solids from the upper to the low bay. Temperature decreased significantly from the upper to the lower bay in all seasons except winter. Because of freshwater input and associated nutrients, nutrient concentrations decreased significantly with increasing salinity from the upper to the lower bay.

### 3.2. Current size-fractionated phytoplankton biomass (chlα)

Total and size-fractionated chlα concentrations in XSB varied significantly \((p < 0.01)\) among seasons (Table 1). The average total chlα concentrations were 2.50 ± 1.64, 2.35 ± 1.49, 1.83 ± 2.29, and 1.41 ± 0.44 \(\mu\)g L\(^{-1}\) in summer, autumn, winter, and spring, respectively. Notably, phytoplankton bloomed frequently in Tie and Huangdun Harbors, particularly in winter (Fig. 3). Pico-chlα concentrations were significantly \((p < 0.05)\) higher in summer \((0.38 ± 0.29 \mu g L^{-1})\) and autumn \((0.37 ± 0.34 \mu g L^{-1})\) than in winter \((0.18 ± 0.25 \mu g L^{-1})\) and spring \((0.10 ± 0.07 \mu g L^{-1})\), micro-chlα concentrations were significantly \((p < 0.05)\) higher in winter \((0.60 ± 1.37 \mu g L^{-1})\) and autumn \((0.67 ± 0.61 \mu g L^{-1})\) than in spring \((0.44 ± 0.12 \mu g L^{-1})\) and summer \((0.27 ± 0.21 \mu g L^{-1})\), and nano-chlα concentrations were significantly \((p < 0.05)\) higher in summer \((1.85 ± 1.32 \mu g L^{-1})\) than in other seasons (Table 1).

The sectional distribution of all size-fractionated chlα was upper > middle > lower (Table 2). The total chlα concentration varied significantly \((p < 0.01)\) among sections in all seasons except summer. Fig. 3 shows that the total and size-fractionated chlα decreased obviously from the upper to lower bay in all seasons. Notably, total chlα concentrations were apparently higher in the three branched bays than in other regions. For example, total chlα concentrations were considerably higher in Tie \((2.64 \mu g L^{-1})\), Huangdun \((5.84 \mu g L^{-1})\), and Xihu Harbors \((3.42 \mu g L^{-1})\) than in other regions \((1.56 \mu g L^{-1})\) in summer.

### 3.3. Current phytoplankton size structure and pheo/chlα ratio

Overall, nano-chlα was overwhelmingly dominant (>55%) in all seasons, whereas pico-chlα contributed only <16% to total chlα (Fig. 4). Phytoplankton size structure varied significantly \((p < 0.001)\) among seasons. The contribution of micro-chlα was significantly \((p < 0.05)\) higher in cold than in warm seasons, whereas contributions of nano- and pico-chlα showed the opposite results (Fig. 4). Pheo/chlα was considerably higher in cold than in warm seasons (Table 1).

Phytoplankton size structure (Fig. 4) and pheo/chlα (Table 2) varied significantly \((p < 0.001)\) among sections. The micro-chlα contribution (except in winter) and pheo/chlα increased with decreasing pico-chlα contribution from the upper to the lower bay in all seasons (Figs. 3 and 4; Table 2). In winter, the micro-chlα contribution was significantly \((p < 0.05)\) higher in the upper (43.1%) than in the middle (14.1%) and lower (14.0%) bay. Additionally, a high micro-chlα contribution was observed in the branched bays (Fig. 3).

### 3.4. Spearman’s rank correlation

The concentrations of nano-, pico-, and total chlα were positively and significantly \((p < 0.01)\) correlated with transparency, temperature, DIP, DSi, Si/N, and mesozooplankton (mainly copepods) abundance and negatively and significantly \((p < 0.01)\) correlated with suspended solids, salinity, and N/P (Table 3). However, pheo/chlα was positively and significantly \((p < 0.01)\) correlated with suspended solids, salinity, and N/P and negatively and significantly \((p < 0.01)\) correlated with transparency, nutrients, and mesozooplankton abundance.

The micro-chlα contribution was positively and significantly
(p < 0.05) correlated with suspended solids and salinity and negatively and significantly (p < 0.01) correlated with transparency, temperature, nutrients, Si/N, and mesozooplankton abundance (Table 3). The pico-chla contribution exhibited the opposite results, whereas the nano-chla contribution showed nonsignificant correlation with environmental variables.

3.5. Long-term changes in temperature and nutrients

The annual (Fig. 5a) and summer SST (Fig. 5b) in XSB increased nonsignificantly by 0.71 and 0.21 °C from 2003 to 2017 with a rate of 0.47 and 0.14 °C decade\(^{-1}\), respectively, according to Mann-Kendall test and Sen’s slope estimation. However, the winter SST increased significantly (p = 0.07) by 1.44 °C with a rate of 0.96 °C decade\(^{-1}\) (Fig. 5c). Fig. 6 shows that concentrations of DIN and DIP increased gradually from 1981/1982 to 2015. However, seasonal average N/P increased sharply from 23.2 in 1981/1982 to 66.3 in 1992 and then decreased (Fig. 6c).

3.6. Long-term changes in phytoplankton biomass and size structure

Table 4 shows that phytoplankton size structure has evidently changed in all seasons since 1992. The annual average contribution of micro-chla increased from 20.6% in 1992 and 15.1% in 2000 to 26.0%
in 2015 and 33.6% in 2017. However, if we exclude spring data because of severe algal blooms in 1992 (Fig. 7a), the micro-chla contribution doubled from 1992 to 2017. Fig. 8 shows that the micro-chla contributions in all sections increased significantly ($p < 0.05$) from 2000 to 2017 in all seasons except summer. Phytoplankton biomass and primary productivity also increased since the 1980s (Fig. 7).

### 4. Discussion

#### 4.1. Seasonal variations in phytoplankton biomass and size structure

The phytoplankton biomass (total chla concentration) in XSB was significantly ($p < 0.05$) higher in warm than in cold seasons (Table 1). This finding was highly associated with significant ($p < 0.01$) seasonal variations in transparency (light availability), temperature, DIN, and DSI. Early studies have suggested that phytoplankton is usually limited by light, nutrients, and turbulence in coastal waters (Margalef, 1978; Cloern, 1987; Domingues et al., 2015; González-Garcia et al., 2018). Compared with cold seasons, intrusion of the clear, offshore Taiwan Warm Current, higher solar radiation, and lower wind-induced mixing in warm seasons produced higher transparency and lower suspended solids, resulting in higher light penetration in XSB. Additionally, nutrient concentrations, particularly DIN and DSI (Table 1), increased significantly in warm seasons because of riverine inputs (Jiang et al., 2013b; NMB, 2016). Table 2 reveals that the total chla concentration was positively and significantly ($p < 0.01$) correlated with transparency, temperature, nutrients, and Si/N and negatively and significantly ($p < 0.05$) correlated with suspended solids, salinity, and N/P. Pheo/chla was lower in warm than in cold seasons, indicating lower algal mortality/senescence in warm seasons (Taylor et al., 2012; Chen et al., 2015). Therefore, phytoplankton in warm seasons benefited from this suitable environment characterized by high light penetration, abundant nutrients, and relatively lower N/P and higher Si/N. Moreover, thermal discharge from power plants evidently elevated SST in the inner bay (Jiang et al., 2019), which stimulated phytoplankton growth and resulted in an increase in average chla of the entire bay, particularly in cold seasons (Fig. 3; Table 2). Considering nutrients were still abundant enough to support algal growth in cold compared with warm seasons (Table 1), we speculated that seasonal change of phytoplankton biomass in XSB was largely regulated by light availability and temperature under variations of solar radiation, vertical mixing, and current.

Although the phytoplankton size structure in XSB exhibited significant ($p < 0.001$) seasonal variation, micro- and nanophytoplankton were responsible for most (>84%) total chla in all seasons (Figs. 3 and 4). This finding was consistent with that of other studies in eutrophic coastal waters (Ho et al., 2010; Guenthner et al., 2015; Oseji et al., 2018). Overall, phytoplankton biomass was dominated (>55%) by the nano-sized fraction, which was consistent with universal size-fractionated results in productive regions and attributable to its competitive advantage in nutrient acquisition and conversion (Marañón, 2015). In cold seasons, the influence of the turbid Zhemin Coastal Current, a turbulent water column, and low transparency were more conducive to large-celled than small-celled phytoplankton (Key et al., 2010; Cloern, 2018), resulting in higher contributions of micro- and nano-chla in cold than in warm seasons (Fig. 4). However, high temperature, water column stability, and transparency favored picophytoplankton (Agawin et al., 2000; González-Garcia et al., 2018), resulting in significantly ($p < 0.05$) higher pico-chla concentrations (Table 1) and contributions (Fig. 4) in warm than in cold seasons. Table 3 confirms this contrasting correlation of micro- and pico-chla contributions with environmental parameters. Mesocosm experiments (Sommer et al., 2012; Peter and Sommer, 2015) and field investigations (Taylor et al., 2012) have demonstrated that small-celled phytoplankton benefit from warming conditions. These findings suggested that seasonal change of phytoplankton size structure was largely regulated by water column stability and temperature. Additionally, significantly ($p < 0.05$) higher

#### Table 3

Spearman’s rank correlation coefficients ($r$) between size-fractionated chla (concentration and contribution) and environmental variables in XSB. Tran: transparency; SS: suspended solids; Temp: temperature; ZooA: mesozooplankton abundance after log-transformation. Micro-, nano-, and pico-chla, respectively, to total chla.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tran</th>
<th>SS</th>
<th>Temp</th>
<th>Salinity</th>
<th>DIN</th>
<th>DIP</th>
<th>DSI</th>
<th>N/P</th>
<th>Si/N</th>
<th>ZooA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-chla</td>
<td>$-0.01^{**}$</td>
<td>$0.08^{**}$</td>
<td>$0.01^{**}$</td>
<td>$-0.11^{**}$</td>
<td>$0.04^{**}$</td>
<td>$0.07^{**}$</td>
<td>$0.01^{**}$</td>
<td>$-0.25$</td>
<td>$0.03^{**}$</td>
<td>$-0.10^{**}$</td>
</tr>
<tr>
<td>Nano-chla</td>
<td>$0.48^{***}$</td>
<td>$-0.40^{**}$</td>
<td>$0.45^{**}$</td>
<td>$-0.71^{**}$</td>
<td>$0.55^{***}$</td>
<td>$0.68^{**}$</td>
<td>$0.53^{**}$</td>
<td>$-0.51^{**}$</td>
<td>$0.39^{**}$</td>
<td>$0.32^{**}$</td>
</tr>
<tr>
<td>Pico-chla</td>
<td>$0.78^{***}$</td>
<td>$-0.70^{***}$</td>
<td>$0.47^{***}$</td>
<td>$-0.73^{***}$</td>
<td>$-0.52^{***}$</td>
<td>$0.79^{**}$</td>
<td>$0.62^{**}$</td>
<td>$-0.62^{**}$</td>
<td>$0.39^{**}$</td>
<td>$0.45^{**}$</td>
</tr>
<tr>
<td>Total chla</td>
<td>$0.52^{***}$</td>
<td>$-0.42^{***}$</td>
<td>$0.44^{***}$</td>
<td>$-0.69^{***}$</td>
<td>$0.48^{***}$</td>
<td>$0.69^{**}$</td>
<td>$0.52^{**}$</td>
<td>$-0.57^{**}$</td>
<td>$0.32^{**}$</td>
<td>$0.30^{**}$</td>
</tr>
<tr>
<td>Pheo/chla</td>
<td>$-0.71^{***}$</td>
<td>$0.68^{***}$</td>
<td>$-0.10^{**}$</td>
<td>$0.51^{***}$</td>
<td>$-0.35^{**}$</td>
<td>$-0.63^{***}$</td>
<td>$-0.35^{***}$</td>
<td>$0.56^{***}$</td>
<td>$-0.17^{**}$</td>
<td>$-0.40^{**}$</td>
</tr>
<tr>
<td>Micro-cont</td>
<td>$-0.37^{***}$</td>
<td>$0.40^{***}$</td>
<td>$-0.37^{***}$</td>
<td>$0.40^{***}$</td>
<td>$-0.33^{**}$</td>
<td>$-0.33^{**}$</td>
<td>$-0.44^{**}$</td>
<td>$0.17^{**}$</td>
<td>$-0.27^{**}$</td>
<td>$-0.34^{**}$</td>
</tr>
<tr>
<td>Nano-cont</td>
<td>$-0.10^{**}$</td>
<td>$0.01^{**}$</td>
<td>$0.11^{**}$</td>
<td>$-0.07^{**}$</td>
<td>$0.12^{**}$</td>
<td>$-0.03^{**}$</td>
<td>$0.13^{**}$</td>
<td>$0.11^{**}$</td>
<td>$0.05^{**}$</td>
<td>$0.11^{**}$</td>
</tr>
<tr>
<td>Pico-cont</td>
<td>$0.80^{***}$</td>
<td>$-0.70^{***}$</td>
<td>$0.38^{***}$</td>
<td>$-0.58^{**}$</td>
<td>$0.40^{***}$</td>
<td>$0.67^{***}$</td>
<td>$0.55^{***}$</td>
<td>$-0.52^{***}$</td>
<td>$0.36^{**}$</td>
<td>$0.43^{**}$</td>
</tr>
</tbody>
</table>

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$. 

---

Fig. 4. Contribution of sized-fractionated chla (micro, nano, and pico) in different XSB sections in all seasons. Different lowercase letters indicate a significant ($p < 0.05$) sectional difference in the given season.
mesozooplankton abundance in warm than in cold seasons (Du, 2018) might be partially responsible for the relatively high pico-chla contribution under high grazing on microphytoplankton and microzooplankton (Sommer et al., 2012; Oseji et al., 2018). Table 3 shows that the micro-chla contribution was negatively and significantly ($p < 0.001$) correlated with mesozooplankton abundance, whereas pico-chla contribution showed the opposite correlation, similarly indicating a high rate of mesozooplankton grazing on microphytoplankton.

### 4.2. Spatial changes of phytoplankton biomass and size structure

Phytoplankton biomass in XSB varied spatially along gradients of transparency, nutrients, and water exchange (Figs. 2 and 3; Table 2). The total and size-fractionated chla in XSB decreased from the stable, clear, eutrophic upper bay to the turbulent, turbid lower bay in all seasons. Because of strong wind and tidal mixing in the lower bay, high sediment resuspension and low transparency occurred (Fig. 2), and thereby severe light attenuation was observed there. Additionally, high nutrient concentrations were found in the upper bay (Fig. 2), because of mariculture and riverine inputs (Jiang et al., 2013a; Yang et al., 2018) and long water-residence time (Ning and Hu, 2002). Thermal discharge resulted in temperature increase in the upper bay, thus stimulating algal growth and even algal bloom (Jiang et al., 2012, 2013b). For example, phytoplankton (mainly *Skeletonema* spp. and *Chaetoceros curvisetus*) bloomed in Tie Harbor in winter (Jiang et al., 2019), with chla concentration up to 10μgL$^{-1}$ (Fig. 3). Previous studies have identified a similar sectional difference in total chla in this bay (Liu et al., 1997, 1998; Ning and Hu, 2002). Table 3 illustrates that pheo/chla was negatively and significantly ($p < 0.01$) correlated with nutrients and transparency, indicating higher algal mortality/senescence in the lower than in the upper bay under strong sediment resuspension and severe light limitation (Chen et al., 2015). This finding supports the spatial distribution of chla concentration found in our study. We inferred that spatial pattern of phytoplankton biomass in the hypereutrophic XSB was largely limited by water column stability and light availability.

Phytoplankton size structure in XSB also showed significant ($p < 0.001$) spatial changes along environmental gradients (Figs. 2–4). Overall, micro-chla contribution increased with increasing suspended solids and with decreasing water-residence time, transparency, temperature (except in winter), and nutrients from the upper to the lower bay in all seasons, but pico-chla contribution exhibited the opposite correlations (Table 3). In winter, however, the bloom of chain-formed diatoms (Jiang et al., 2019) induced by thermal discharge in the upper bay resulted in a predominance of micro-chla (Fig. 3). According to the principle of phytoplankton cell-size tradeoffs, large cells benefit from a turbulent, turbid, eutrophic environment (Litchman et al., 2007; Key et al., 2010; Cloern, 2018), whereas small cells prefer a clear, warm, oligotrophic, stable water column (Agawin et al., 2000; González-

### Table 4

Long-term variation in micro-chla contribution (%) to total chla in XSB.

<table>
<thead>
<tr>
<th>Year</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Annual average</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992</td>
<td>15.0</td>
<td>31.7</td>
<td>11.4</td>
<td>24.2</td>
<td>20.6</td>
<td>Liu et al., 1997</td>
</tr>
<tr>
<td>1994</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>14.0</td>
<td>nd</td>
<td>Liu et al., 1998</td>
</tr>
<tr>
<td>2000</td>
<td>24.0</td>
<td>8.0</td>
<td>17.2</td>
<td>11.4</td>
<td>15.1</td>
<td>Ning and Hu, 2002</td>
</tr>
<tr>
<td>2015</td>
<td>33.1</td>
<td>31.5</td>
<td>10.9</td>
<td>28.4</td>
<td>26.0</td>
<td>This study</td>
</tr>
<tr>
<td>2017</td>
<td>34.7</td>
<td>47.6</td>
<td>26.5</td>
<td>25.7</td>
<td>33.6</td>
<td>This study</td>
</tr>
</tbody>
</table>

**nd**: No data.
Moreover, small cells with relatively high buoyancy have a competitive advantage over large cells under reduced water density and vertical mixing (Winder and Sommer, 2012). Therefore, we suggested that phytoplankton size structure in XSB on a spatial scale was largely shaped by water column stability and light availability associated with vertical mixing rather than by nutrients, in addition to the anthropogenic thermal effect.

4.3. Long-term variations of phytoplankton biomass and size structure

Phytoplankton biomass and primary productivity in XSB have increased sharply since the 1980s (Fig. 7). We inferred that this trend is closely related to increased warming (Fig. 5) and eutrophication (Fig. 6). Although this study did not examine a warming trend in XSB since the 1980s, the summer SST in adjacent Changjiang Estuary increased by 1.31 °C from 1982 to 2009 (Jiang et al., 2014), possibly indicating rapid warming in XSB as well under climate change. Moreover, thermal discharge accelerated the warming trend in XSB since 2006 because of long water-residence time (Fig. 5; Jiang et al., 2013b, 2019). Similarly, algal blooms and chla concentration in Daya Bay have gradually increased because of nutrient enrichment and thermal effluent from a nuclear power station (Yu et al., 2007; Wu et al., 2017). Mesocosm experiments and field investigations have confirmed that a modest temperature increase is conducive to algal growth and productivity (Jiang et al., 2012, 2013b; Yvon-Durocher et al., 2015; Xie et al., 2018). Under accelerated warming at a rate of 0.96 °C decade⁻¹ in winter (Fig. 5c), algal bloom seasonality in XSB has shifted from spring to winter since the power plant operations in 2006 (You and Jiao, 2011; Jiang et al., 2013b, 2019). Particularly in the upper bay, phytoplankton bloomed frequently under thermal stimulation (Fig. 3; Jiang et al., 2012). Earlier timing of the spring bloom under climate warming was also widely observed in other marine systems (Winder and Sommer, 2012). Furthermore, primary productivity in winter increased remarkably from 1992 to 2007 (Fig. 7b). Several studies have emphasized that climate warming has resulted in earlier algal bloom and production and may profoundly influence pelagic food webs such as match–mismatch (Hallegraeff, 2010; Anderson et al., 2012), showing a different phenology response between phytoplankton and zooplankton. Thus, thermal addition from power plants has probably altered the food-web structure in XSB, which warrants further study.

The contribution of micro-chla increased remarkably from 1992 to 2017 (Table 4), which was highly associated with eutrophication (Fig. 6) and warming (Fig. 5). The net-collected (large-celled) phytoplankton abundance in XSB also increased with increasing temperature and DIN and DIP concentrations (Jiang et al., 2013b), thus supporting this finding. Large cells such as diatoms and dinoflagellates have a competitive advantage over small cells in nutrient-rich coastal waters because of different adaptation to nutrients (Stolte and Riegman, 1995; Cermeño et al., 2005; Irwin et al., 2006; Marañón et al., 2007). However, studies have demonstrated that temperature increases reduced phytoplankton cell size through increased metabolic rates and consequent resource competition and predator–prey interactions (Sommer et al., 2012; Taylor et al., 2012; Peter and Sommer, 2013, 2015). These results, contrasting with those of our study, may be attributed to dominance of the nutrient effect over the temperature effect (Peter and Sommer, 2013, 2015). Marañón (2015) suggested that biogeographic patterns in phytoplankton size structure are largely driven by resource supply rather than by temperature, thus supporting our assumption. A 24-h laboratory experiment determined that the micro-chla contribution in Daya Bay, South China Sea decreased in summer and increased in winter after a 4°C rise in temperature and nutrient addition (Xie et al., 2018), which was probably caused by a temperature increase up to 32°C in summer that exceeded the thermal tolerance of microphytoplankton. Our study also observed that a large-celled phytoplankton bloom in winter enhanced the micro-chla contribution under thermal addition (Fig. 3).

Additionally, different effects of nutrient limitations and zooplankton grazing on size structure in different sites may also be

Fig. 7. Long-term variations in chla and primary productivity in XSB. Data from the literatures (ECBCC, 1992; Liu et al., 1997, 1998; Ning and Hu, 2002; Huang et al., 2008; You and Jiao, 2011; Jiang et al., 2013b; Wang et al., 2013; Shou and Zeng, 2015).

Fig. 8. Comparison of micro-chla contributions in the different sections (upper, middle, and lower) before (2000; Ning and Hu, 2002) and after (2015 and 2017) the start of power plant operations using the Kruskal–Wallis test. Different lowercase letters indicate a significant (p < 0.05) difference among years in the given section.
responsible for the aforementioned debatable consequence of eutrophication and warming. In a mesocosm experiment, Peter and Sommer (2015) found that the negative effect of temperature on cell size was strongest under N limitation, intermediate under P limitation, and weakest when N and P were balanced at a N/P of 16. Moderate P limitation at a N/P > 28 in XSB (Fig. 2h) might reduce the negative effect of temperature increase on phytoplankton cell size. Field observation in Daya Bay, South China Sea revealed that the micro-chla contribution increased with increasing temperature in P-limited waters that received thermal discharge (Xie et al., 2018). Sommer et al. (2012) demonstrated that phytoplankton cell size under warming responded negatively to copepod density, indicating positive mesozooplankton grazing on microphytoplankton. A 5-year outdoor mesocosm experiment showed that warming favored larger phytoplankton taxa because of resistance to zooplankton grazing, suggesting stronger top-down control and trophic interactions (Yvon-Durocher et al., 2015). Moreover, Chen et al. (2012) determined that warming enhanced microzooplankton grazing on phytoplankton in eutrophic waters, suggesting that the microphytoplankton contribution increased under predator–prey interactions. Agawin et al. (2000) found a decrease in the picophytoplankton contribution in warm productive or eutrophic waters and attributed it to increased loss rates such as strong grazing pressure. Table 3 shows that micro-chla contribution was negatively and significantly (p < 0.001) correlated with mesozooplankton abundance, whereas pico-chla contribution showed the opposite correlation. We inferred that increased microzooplankton grazing on small-celled phytoplankton probably resulted in an increased microphytoplankton contribution.

Another reason for micro-chla contribution increase in XSB might be the increased dominance and HAB occurrence of large-celled dinoflagellates (You and Jiao, 2011; Jiang et al., 2013b) under accelerated warming and eutrophication (Fig. 7). Dinoflagellates benefit from a habitat with high nutrients, N/P, and temperature because of ecophysiological tradeoffs (Smayda, 1997; Finkel et al., 2010; Xie et al., 2015). Our microscope observations in 2015 (Jiang et al., 2015) and 2016–2019 (unpublished data) revealed that certain large-celled dinoflagellate species including S. trochoidea, Akashiwo sanguinea, Gymnodinium catenatum, and Karenia mikimotoi were dominant. However, dinoflagellates were not dominant in 2000 (Ning and Hu, 2002) or 2006/2007 (Shou and Zeng, 2015). Similarly, our long-term observations in Changjiang Estuary revealed that dinoflagellate dominance increased with increasing nutrients, N/P, and SST (Jiang et al., 2014). In Daya Bay, increased eutrophication and temperature also resulted in greater dominance of large-celled dinoflagellates, including genera Scripsiella, Ceratium, Protoperidinium, and Dinophysis (Li et al., 2011; Wu et al., 2017). Moreover, HABs caused by flagellates, mainly S. trochoidea, A. sanguinea, and Chattonella marina, have increased sharply since 2000 (Yu et al., 2007; Wu et al., 2017). Xiao et al. (2018) predicted that dinoflagellate blooms in East China Sea would become more frequent and intense with increasing SST and eutrophication, using statistical niche models based on field-observed data. Coastal nutrient enrichment will promote large-celled algal blooms and thereby a shift toward larger mean cell size, regardless of warming (Marañón, 2015).

5. Conclusions

This study partially confirmed our hypothesis that phytoplankton biomass and size structure in XSB had highly significant spatial and seasonal variations, which were largely shaped by water column stability and light availability rather than by nutrients. Additionally, temperature increase caused by thermal discharge frequently stimulated algal bloom in the upper bay and profoundly influenced microchla contribution. Since the 1980s, phytoplankton biomass, primary productivity, and micro-chla contribution have apparently increased with increasing nutrients and temperature, although a quantitative relationship has been difficult to ascertain because time-series data are lacking. In such conditions, food webs become shorter, which may profoundly influence local trophic structure and carbon flow. This shift in phytoplankton cell size should be taken into account in future predictive models. Furthermore, our study suggested that the temperature increase caused by thermal discharge was useful in simulating the potential rapid climate warming effects on phytoplankton. This paper provides preliminary insight into the current state and long-term variations of phytoplankton biomass and size structure in XSB, which may be useful in determining the potential ecological consequences such as food-web dynamics and biogeochemical processes due to increased eutrophic warming and warming in coastal ecosystems worldwide.

Acknowledgments

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